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TITLE: Potential Therapeutic Use of Glyceollins (I-III), Novel Anti-Estrogenic Flavonoid Phytochemicals Isolated from Soy

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13. ABSTRACT (Maximum 200 Words) The flavonoid family of phytochemicals, particularly those derived from soy, has received attention regarding their estrogenic activity as well as their effects on human health and disease. The types and amounts of these compounds in soy and other plants are controlled by both constitutive expression and stress-induced biosynthesis. The health benefits of soy may therefore be dependent upon the amounts of the various hormonally active phytochemicals present. We have identified increased biosynthesis of the isoflavonoid phytoalexin compounds, glyceollins I, II and III, in soy plants grown under stressed conditions (elicited soy) which exhibit marked anti-estrogenic effects on ER function. The glyceollin (I-III) compounds may represent an important component of the health effects of soy as well as represent novel anti-estrogens useful in the prevention or treatment of breast carcinoma. In this grant we propose to test the hypothesis that elicited soy extract, which has been shown to be anti-estrogenic in cell culture, is therapeutic in a tumor-transfer model of breast cancer. Using this model, we additionally hypothesize that specific Glyceollins, isolated from elicited soy, will display anti-estrogenic activity <i>in vivo</i> , suppressing the growth of breast tumors. To this end in the first year of this proposal we have, 1) completed preliminary studies establishing the MCF-7 <i>in vivo</i> immunocompromised mouse model of tumorigenesis in this lab; 2) generated and 110 Kg of elicited soybeans to be converted to protein for use in the mouse soy diet studies and confirmed Glyceollin expression in various products; 3) begun separation of individual Glyceollins(I,II,III) from purified Glyceollin (I,II,III) mixture; 4) established the <i>in vitro</i> suppression of colony formation (survival/proliferation) by Glyceollins; and 5) demonstrated suppression of estrogen stimulated VEGF expression by Glyceollins.		
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INTRODUCTION

Flavonoids represent a family of phytochemicals that function to deter herbivores, act as antibacterial/antifungal agents and stimulate formation of symbiotic relationships with nitrogen-fixing bacteria [23,28,36]. Although the function of these compounds is not completely understood, they affect not only bacteria and fungi but have been described to exert effects on mammals as well [15,23,28,36,40]. The observation that sheep grazing on fields rich in clover and cheetahs fed high soy diets in zoos had reproductive anomalies demonstrated that flavonoids and related phytochemicals can affect mammalian health [4,38,40]. Of interest was the observation that they function as estrogenic mimics or phytoestrogens and may represent important dietary factors affecting human health [5,11,25,32,42]. The estrogenic phytochemicals which include flavonoids, lignans, phytostilbenes and enterolactones, appear to primarily function by binding to and activating the estrogen receptor (ER), albeit at 100-1000 greater concentrations than 17 β -estradiol [12,13,20,30,31]. Two key constitutive isoflavonoids most often detected in soybean tissue, genistein and daidzein, have been widely examined for these effects. The observation that soy phytochemicals can function as ER agonists is consistent with the observed health benefits of soy foods such as decreased incidence of osteoporosis and cardiovascular disease [1,2,5,11,19,25,32,33,42,45]. However, the similar decrease in risk of breast cancer would indicate a potential antiestrogenic activity of soy phytochemicals [1,12,13,19,45]. Additionally, the ability of soy isoflavonoids to prevent carcinogen-induced mammary tumorigenesis further implicates potential antiestrogenic effects of these compounds. Consistent with this information, certain phytochemicals have been reported to exert antiestrogenic effects at higher concentrations [12,13]. These studies however were not exclusive to soy-derived isoflavonoids suggesting that many flavonoids may function both as ER agonists or antagonists in dose and cell type specific manner. The recent identification of a second estrogen receptor beta (ER β) with different affinity for and transactivation by phytoestrogens represent another mechanism by which flavonoids may function to regulate estrogen signaling [18,26,27]. The suggestion that the high isoflavonoid content of soy may function to prevent cancer and disease is bolstered by the observation that the predominant isoflavonoids found in soy, genistein and daidzein, can affect estrogen signaling and prevent cancer in animal models. However, genistein and daidzein represent only two compounds in the complex flavonoid biosynthetic pathway, and the amount and type of isoflavonoid present in soy can be readily altered in response to external stimuli, thus illustrating that other isoflavonoids must be considered in relation to the health effects of soy products. Additionally, environmental factors and growth conditions can alter the biosynthesis leading to production of numerous flavonoids that have not been characterized for their effects in mammalian systems [14,17,21]. Given that the biosynthesis of isoflavonoids can be regulated by external factors, the type and amount of hormonally active phytochemicals may vary from source to source. Additionally, the environmentally induced biosynthesis of unique isoflavonoids of undefined hormonal activity may represent an important component of beneficial effects of these compounds on human health.

Previous studies in our laboratories identified unique soy phytochemicals that had not been assessed previously for estrogenic or antiestrogenic activity [6,8,34]. The soybean isoflavonoids, glyceollins I-III, were elicited by the fungus *A. sojae*, a non-toxin producing *Aspergillus* strain commonly used in the fermentation of soybeans to produce soy sauce and miso, and isolated as a group (isomers I-III) from the extract of the elicited soy. The glyceollin isomers I-III have similar core structures to that of coumestrol and are derived from the precursor daidzein in the glyceollin pathway. The ability of the elicited soy extract and the glyceollins to regulate estrogen signaling was analyzed using the ER positive MCF-7 human breast carcinoma cell line and the ER negative HEK 293 cells transfected with either ER α or ER β [8]. Elicited soy extract had reduced estrogenic activity compared to normal (non-elicited) soy extract, and somewhat surprisingly, exhibited moderate anti-estrogenic activity in the presence of E2. While the glyceollins isolated from the elicited soy extract displayed

negligible estrogenic activity, they did display a dose dependent suppression of 17 β -estradiol induced transactivation and MCF-7 cell proliferation. The glyceollins also functioned to suppress estrogen activity through both ER α and ER β which correlated with binding respectively to either ER α or ER β . All of these experiments used a mixture of glyceollin isomers, so their individual estrogen binding and signaling characteristics are still unknown. Neither elicited soy extracts nor glyceollins have been tested *in vivo*, but we would predict anti-estrogenic action based on these *in vitro* results.

In vitro assays are useful screening tools, but can be incomplete for predicting *in vivo* results. One difficulty in translating *in vitro* results to *in vivo* effects is that the complement of active signaling pathways, response elements, co-activators and co-repressors can differ among species or tissues, and even in different cell types within a tissue [16]. Classical *in vivo* tests of estrogenicity include assays of uterine proliferation and of nuclear progesterone receptor (PR) content. While E2 causes growth in rat uterine weight and increases in PR densities, phytoestrogen effects vary with compound and age of subject, being more effective at producing uterotrophic effects in immature females than in adults [7,35,41,43,44]. Some of the inconsistencies of *in vivo* effects of soy extracts might relate to differing complements of phytoestrogens, some being estrogenic and others anti-estrogenic, or may depend on developmental differences in ER subtypes or their relative densities.

Similar to studies on effects of soy extracts on the uterus, the direction of the effect on breast tissue may depend on the developmental state of the animal involved [3] and possibly on the particular phytoestrogens used in the studies. In some studies, soy isoflavonoids have been chemopreventive in xenograft or carcinogen-induced animal models of breast cancer. For the most part, these studies have exposed animals to phytoestrogens neonatally or pre-pubertally, potentially influencing the timing of mammary tissue development (reviewed by Whitten and Patisaul [43]). Other studies, however, have demonstrated estrogen-like effects of phytochemicals on normal breast tissue or growth of tumors in animal models [43]. Pre-menopausal women ingesting soy protein (containing 38mg isoflavone) daily for several months showed signs of estrogen-like stimulation of breast tissue: increased nipple aspirate fluid volume and appearance of hyperplastic epithelial cells [37]. No effects of treatment were found in postmenopausal women in the same study. Adult rats treated with soy extract (18mg i.p., 5 times/week for 30 days) after receiving mammary tumor cells (MAC-33, s.c.) exhibited increased tumor growth and lung metastasis compared to controls [9], possibly due to an estrogenic effect of the phytoestrogens. Dietary administration of genistein (750ppm) also caused growth of implanted MCF-7 tumors in ovariectomized athymic nude mice [24]. These genistein-stimulated tumors reached the same size as those stimulated by E2 (2 mg pellet, s.c.), but at a much slower rate. In another study using MCF-7 xenografts in athymic nude mice, genistein (0.5mg/kg i.p. every other day) reduced E2-induced tumor growth by 25% compared to E2 alone [39], possibly by acting as a weak agonist in the presence of the strong ER agonist, E2. There was no group without E2 supplementation in this study, so it is unclear whether genistein would have caused slow tumor growth on its own as in the Hsieh et al. [24] study or if tumor growth in the genistein+E2 group would have been significantly different from negative controls. Given the anti-estrogenic effects of elicited soy extract (and glyceollins in particular) *in vitro*, an exciting next step is to determine whether these novel compounds also antagonize effects of E2 *in vivo*, specifically tumorigenic effects of E2 in the athymic nude mouse model.

BODY

We have broken work performed during year one of this proposal into tasks:

Task 1. To purify glyceollins I, II, and III, and determine their effects on ER (α/β) activity, (Months 1-21):

Task 1.A **Isolate and purify glyceollins I, II, and III.** (Months 1-6) A method developed in our laboratory based on fungal inoculation of cut soybean seeds will be utilized for the preparation of glyceollin. Preparative scale HPLC will allow us to isolate the individual glyceollin isomers I, II, and III. Generation of elicited soy extract and glyceollin isolate will continue throughout the project to provide compounds for animal diets

Task 1.B **Determine the estrogenic/antiestrogenic activity of glyceollins I, II, and III using MCF-7 cells transfected with an ERE-Luc plasmid.** (Months 6-18) Given our experience with MCF-7 cells, initial experiments will examine the estrogenic and antiestrogenic activities of glyceollins I, II, and III.

Task 1.D **Determine the effects of glyceollins I, II, and III on the proliferation of ER-positive MCF-7 cells.** (Months 18-21) The proliferation of MCF-7 cells is an established biological response to 17β -estradiol and a useful screening tool for compounds that may function as estrogen agonists or antagonists.

Studies were initiated to separate and isolate individual Glyceollins from a mixture. This is an ongoing effort on this project. We had initially hoped to have separate isolation of Glyceollins 1, 2 and 3 by the end of year one. As can be seen in figure 1 we have only partially separated the individual components. While we have significantly enriched for glyceollin III, the least present glyceollin in elicited soy, glyceollin II remains in this fraction. Once further separated, we will investigate the biologic activity of individual Glyceollin compounds (I,II, III). In light of incomplete separation of individual glyceollins, we have continued to pursue biologic studies with the Glyceollin (I-III) mixture as well as normal and elicited soy extracts. These studies are still highly relevant as we are using isolated fraction of mixed Glyceollins (I-III) in ratios found in stressed soy.

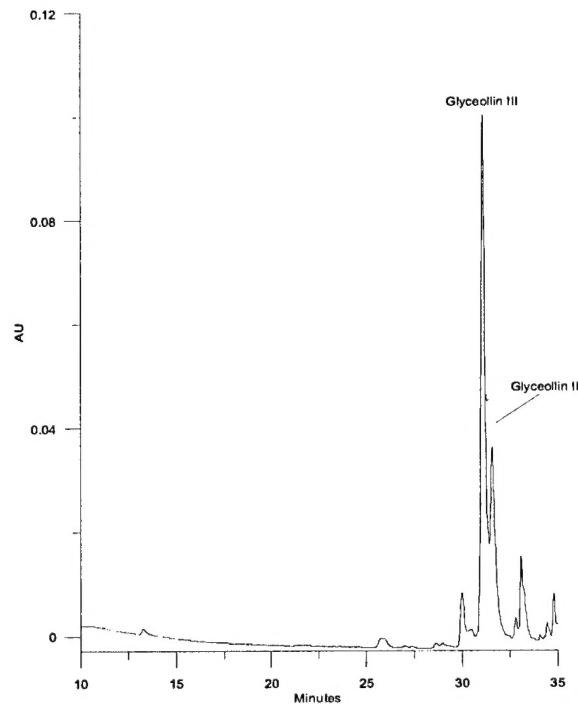


Figure 1. HPLC separation for the isolation of glyceollin III. Second separation needed for complete isolation. HPLC chromatogram at 285 nm.

In our initial publication we demonstrated that Glyceollins exhibited a dose-dependent suppression of proliferation of MCF-7 breast carcinoma cells. These initial studies were performed using the alamar blue proliferation assay, an indirect measure of proliferation (8). Using the Glyceollin mixture we have now demonstrated that the glyceollins can suppress estrogen dependent colony formation of MCF-7 cells (Figure 2). Figure 2 shows the results from an MCF-7 colony assay. The glyceollins alone showed only slight estrogenic activity at 10 μ M. The glyceollins combined with both 0.1 nM and 1 nM 17 β -estradiol significantly inhibited cell growth between 25-50 μ M. The colony assay is a more direct assessment of cell proliferation and viability over time. This data confirms the anti-estrogenic activity of glyceollins in suppression of estrogen-dependent proliferation/survival of ER+ MCF-7 breast carcinoma cells.

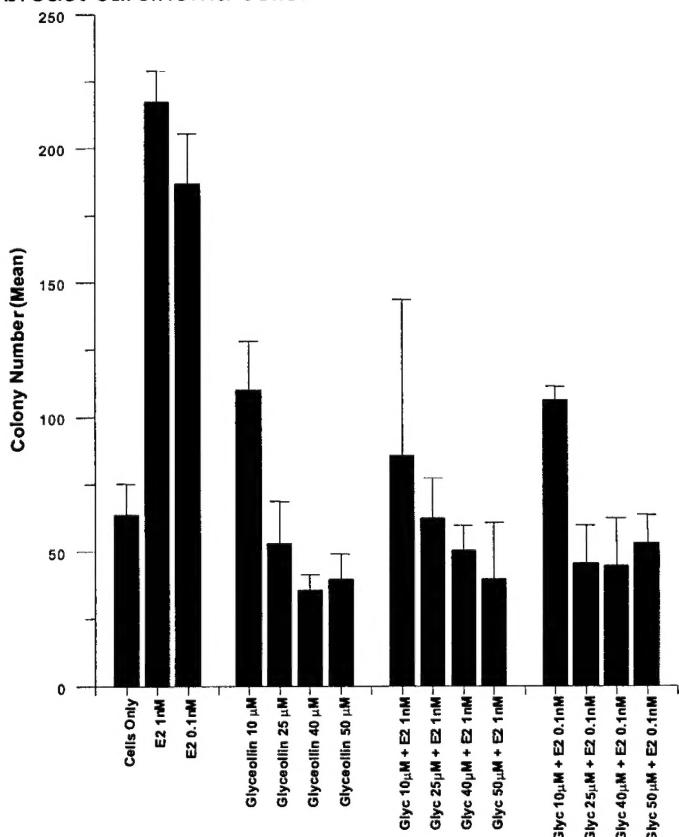


Figure 2. MCF-7 Colony Assay showing antiestrogenic activity of the glyceollins (I-III). MCF-7 cells were plated in 6-well plates at 1000 cells/well. After 24 h the cells were dosed with treatment media. Treatment media (DMEM) consisted of either DMSO, 17 β -estradiol (0.1 or 1.0 nM), glyceollin (I-III) (10, 25, 40, or 50 μ M), or glyceollin combined with 17 β -estradiol. The plates were incubated at 37°C (5 % CO₂) and periodically checked for colony growth of 50 or more cells. After 14 days the cells are fixed with glutaraldehyde for 30 min at 37°C, washed and stained with crystal violet (0.4%) for 1 hr. Colonies are then counted.

While a number of estrogen-responsive genes have been identified that clearly play a role in the biologic effects of estrogens we chose to examine VEGF production as a potential gene product regulated by normal soy vs elicited soy as well as to examine the anti-estrogenic effects of Glyceollins on an endogenous estrogen-regulated gene. VEGF has been demonstrated to be an estrogen responsive gene in MCF-7 cells as well as other ER+ tumor lines. Additionally overexpression of VEGF in MCF-7 cells leads to a hormone independent phenotype similar to our results with MCF-7TNR cells (see below). We used an ELISA based approach to examine estrogen induced production/secretion of VEGF in our hormone dependent MCF-7N and cells (Figure 3). MCF-7 cell lines display an increase in estrogen stimulated VEGF secretion. Interestingly the estrogen induced VEGF production is suppressed by both Glyceollin (25 μ M) and the pure anti-estrogen ICI (100nM) to a similar degree. Normal Soy treatment alone enhanced VEGF production and did not antagonize E2-stimulated VEGF. In contrast Elicited soy 9glyceollin containing did not stimulate VEGF production and slightly antagonized E2 stimulate VEGF production, consistent with elicited soy's previously described antagonistic effects on ER-mediated gene expression.

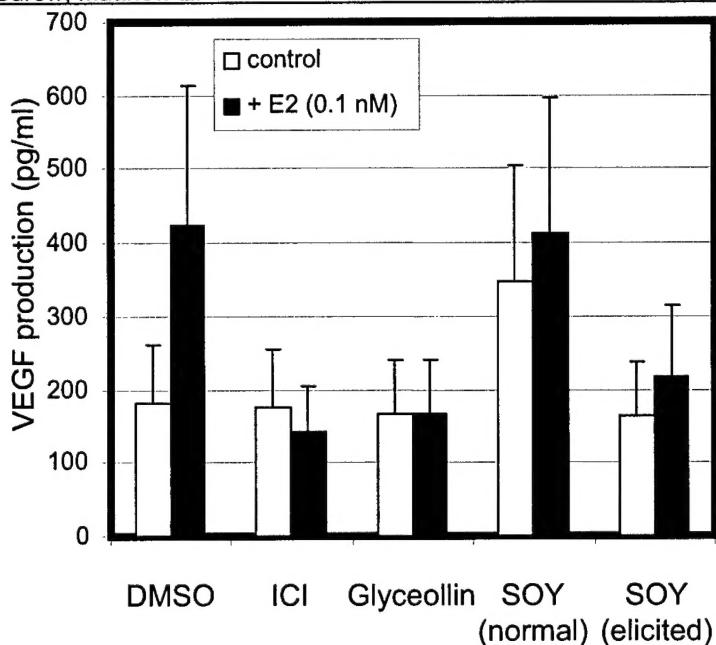


Figure 3. Glyceollin and elicited Soy suppress estrogen-induced VEGF production. MCF-7N cells were plated in 5%CS-DMEM and treated with either DMSO, ICI 182,780 (100 nM), Glyceollin (25 μ M), normal Soy (100 μ g/ml), or elicited Soy (100 μ g/ml) in the presence or absence of estrogen (1nM) for 24 hours. Supernatants were harvested for VEGF ELISA performed according to manufacturers instructions (R&D systems). Data is represented as pg/ml of VEGF as determined from a standard curve \pm S.D. of two experiments.

Task 2 To test the hypothesis that elicited soy extract inhibits growth of implanted breast cancer cell (MCF-7) tumors *in vivo*, (Months 10-20):

Task 2.A Experimental preparation (generate elicited soy extract for diet, grow MCF-7 cells, acclimate animals). (Month 10). Maintain all animals ($n = 48$) on phytoestrogen-free diet during acclimation.

During year one approximately 110Kg of soy beans were elicited and sent for processing into defatted soy protein for use in generation of the soy-protein diets. Initial batches were used to confirm the presence of Glyceollins in the defatted soy protein (Figure 4). This work was performed in collaboration with Dr. Thomas Clarkson in the Center for Comparative Medicine at Wake Forest University. *Soybean treatment and harvesting: Aspergillus sojae* (SRRC 1125) cultures were grown at 25°C in the dark on potato dextrose agar. After 5 days, inoculum was prepared by harvesting conidia (3.4×10^7 /ml) in 15 ml sterile, distilled H₂O. Seeds (1 kg) from commercial soybean variety Asgrow 5902 were rinsed three times with deionized-H₂O. Seeds were presoaked in sterile deionized-H₂O for 4-5 hr. Seeds were cut using a Cuisinart and placed on trays in 1/4 in layer. *A. sojae* spores suspension (200 μ l) was applied to each tray. All chambers were stored at 25° C in the dark for three days, and then transferred to -70 °C. Seeds were rinsed with water to remove spores, filtered, and oven dried at 40 °C. The dried material was processed into prt isolate and several waste streams.

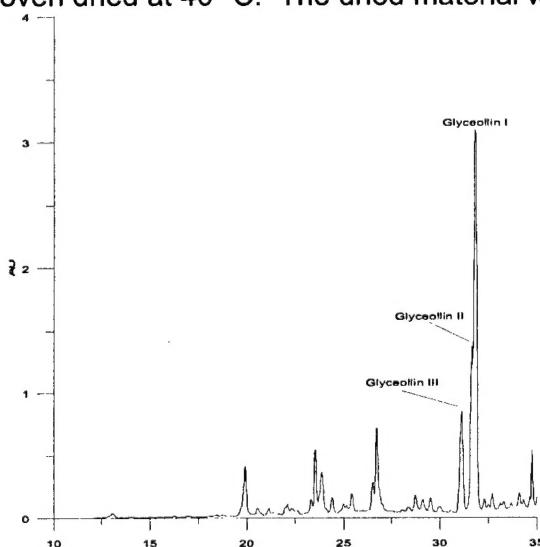


Figure 4. Defatted soy protein isolate using hexane containing a high concentration of glyceollins. HPLC chromatogram at 285 nm.

Task 2.B **Implant MCF-7 cells and E2 pellets into athymic nude mice and measure tumor size weekly for 5-6 weeks.** (Months 11-12) Initiation of MCF-7 tumors requires E2 exposure. Analyze extracts for isoflavone content and prepare experimental diets during this time.

Using an established method of generating drug resistant clones, the ER+, estrogen dependent MCF-7N cell line used in our lab was passaged in increasing concentrations of tumor necrosis factor alpha. A resistant clone was selected and expanded to generate the TNF-resistant MCF-7TNR cell line. These cells exhibited stable resistance to death receptor induced cell death (publication submitted to International Journal of Oncology). Interestingly early studies *in vitro* also demonstrated a resistance to tamoxifen and hormone-independent proliferation (data not shown). Consistent with this, experiments using immuno-compromised mice demonstrate that while the MCF-7-TNR cells for tumors in a hormone-independent manner, they remain estrogen-responsive (Figure 6A). Consistent with the established model, MCF-7N parental cells form tumors only in the presence of exogenous estrogen supplementation in ovariectomized(OVX) -NOD/SCID mice. However the MCF-7TNR cells for tumors in the absence of estrogen the growth of which is increased by estrogen supplementation. We performed these initial studies in ovariectomized NOD-SCID mice to initially establish the technique of growing estrogen stimulated tumors in our lab. This allowed us to establish in our hands baseline parameters for subsequent studies examining Soy as a dietary component in tumor formation or the effects of Glyceollins on tumor growth. In the case of the established clinically used anti-estrogen tamoxifen, in the many patients display early responses to treatment but ultimately their tumors progress to a hormone-independent and anti-estrogen resistant phenotype. WE established the *in vivo* growth of the hormone-independent but estrogen-responsive MCF-7TNR cells as an additional model for use in our studies. This system would allow us to compare the effects of Glyceollins to tamoxifen in an additional system. The potential exists that the pure-antagonistic activity of Glyceollins on ER-mediated gene expression may translate into a new class of anti-estrogens based upon the Glyceollin core structure.

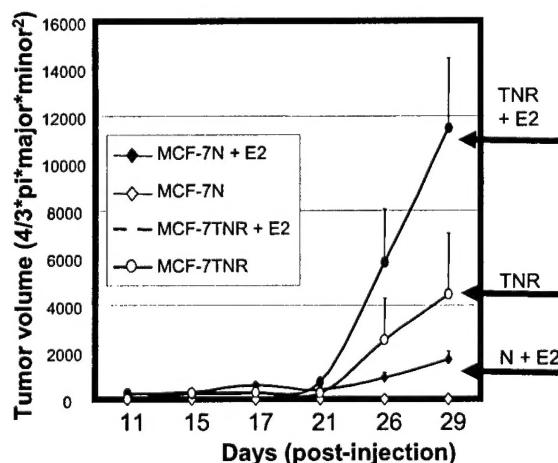


Fig. 5. Establishment of *in vivo* growth of hormone-dependent MCF-7 and hormone-independent (MCF-TNR cells). MCF7N or MCF7-TNR cells (5×10^5) were injected (s.c.) into the flanks of OVX NOD-SCID mice either in the presence (+E2) or absence of slow release estradiol pellets (1.5 mg, 60 day release) (n=6/group). Tumor growth was monitored biweekly after palpable tumor formation and was represented as tumor volume (mm^3) \pm S.E.M.

KEY RESEARCH ACCOMPLISHMENTS

- Initiated separation of Glyceollin isomers
- Established scaled up production of elicited soy bean for protein isolation and soy-diet production
- Further demonstrated anti-proliferation/survival activity of mixed-glyceollins
- Demonstrated the ability of Glyceollins and elicited soy to antagonize estrogen-stimulated VEGF production

REPORTABLE OUTCOMES**Manuscripts in preparation or submitted:**

Boué, S.M., Wiese, T.E., Nehls, S., **Burow, M.E.**, Elliott, S., Carter-Wientjes, C.H., Shih, B.Y., McLachlan J.A., Cleveland, T.E. Evaluation of the estrogenic effects of legume extracts containing phytoestrogens. *J. of Agriculture and Food Chemistry*. 51(8): 2193-2199, (2003).

Presentations:

Department of Medicine Grand Rounds, November 2003, "Hormone Resistance Breast Cancer, Dairy Cows to Drug Development". **Matthew E. Burow**

Comparative Medicine Clinical Research Center, Wake Forest University, and Physicians Laboratories, April 2002 "Glyceollins 1-3, novel anti-estrogenic phytochemicals isolated from stressed soy". **Matthew E. Burow**

E.Hormone2002, Tulane University, Oct 2002 "Cellular Biology of Estrogenic Signaling: A Case for Convergence." **Matthew E. Burow**

Abstracts:

Boué, S.M.; Wiese, T.E.; Carter-Wientjes, C.H.; Shih, B.Y.; **Burow, M.E.**; Wiese, T.E.; and Cleveland, T.E. "Antihomonal Effects of the Soybean Phytoalexin Glyceollin", 5th International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, Sept 21-24, 2003, Orlando, FL.

Boué, S.M.; **Burow, M.E.**; Wiese, T.E.; Shih, B.Y.; Carter-Wientjes, C.H.; Morales, V.S.; McLachlan, J.A.; and Cleveland, T.E. "Antihomonal Effects of the Soybean Phytoalexin Glyceollin", Tulane CBR e.hormone Meeting, October 16-18, 2003, New Orleans, LA.

Stephen M. Boué, Thomas E. Wiese, Suzanne Nehls, **Matthew E. Burow**, Steven Elliot, Carol H. Carter-Wientjes, Betty Y. Shih, and Thomas E. Cleveland. Evaluation of the Estrogenic Effects of Legume Extracts Containing Phytoestrogens, E.Hormone 2002 Conference, at Tulane University, Oct. 17th, 2002-New Orleans, LA

Funding applied for:

"Glyceollin anti-estrogens: parent compounds for the development of novel endocrine agents/hormone therapy"

(Principal Investigator)

Agency: NIH-NCI; Type: rapid access to NCI drug development (RAND); Submitted April 30, 2003. The long-term objective of this proposal is to develop a new class of anti-estrogens based upon the recently identified anti-estrogen flavonoids Glyceollin (I,II,III). The basis for this RAND proposal is the data gained from the currently funded proposal (DAMD-17-02-1-0596) to establish the utility of Glyceollins as novel anti-estrogens. Ultimately the RAND proposal will represent a mechanism by which we can develop the Glyceollins into novel parent drugs for a new class of anti-estrogens. The initial submission was not funded and it was recommended that we further establish the utility of the Glyceollins *in vivo*. The ability to demonstrate the effectiveness of Glyceollins *in vivo* under the current grant will allow us to ultimately pursue a resubmission of the RAND proposal.

Employment opportunities based upon this award**PRINCIPAL INVESTIGATOR**

I received notification of funding of this award as a Research Assistant Professor (non-tenure tracked) in the Department of Pharmacology at Tulane University School of Medicine in December of 2001. In 2001 I began applying for Tenure-track faculty positions at Tulane University Health Sciences Center and at the Louisiana State University Health Sciences Center (LSUHSC). This award allowed me to pursue a novel research project and develop an independent research program. This award further demonstrated my ability to secure funding from a national agency for my research program. As such the funding of this award had a direct positive impact on my ability to secure a Tenure-tracked Assistant Professor position.

Assistant Professor (TenureTrack)
Department of Pharmacology, LSUHSC.

Applied for May 2001
Interviewed September 2001.
Position Offered December 2001.
Position Declined January 2002

Assistant Professor (TenureTrack)
Section of Hematology and Medical Oncology,
Department of Medicine, Tulane University School of Medicine.
Applied for August 2001
Interviewed September 2001.
Position Offered December 2001
Position **Accepted** January 2002

TRAINEES

Christine M. Dugan, M.S

Christine was hired during the first year of this project as a Medical Research Specialist from November 2002 through July 2003. During this period she interviewed for Medical School and was accepted for the fall 2003 class in the M.D./Ph.D. Medical Scientist training program at Michigan State University Medical School.

CONCLUSIONS

The primary goal of the proposed task in year one was to isolate individual Glyceollin components and initiate studies with the individual Glyceollins *in vitro*. The isolation of individual components has been initiated and is ongoing. Given the complications of this process purified Glyceollin (1,2,3) has not been completely achieved to allow us to accurately assess individual compound biologic activity. In lieu of this we have continue our studies using normal and elicited soy (Dietary comparison) and purified Glyceollin (I-III) mixture. These Biologic studies revealed as expected the ability of Glyceollins to suppresses breast carcinoma cell growth (proliferation/viability) as measure by a long-term clonogenicity assay. Additionally we demonstrate that Glyceollins and elicited soy are capable of suppressing estrogen induced VEGF production. Given that VEGF is an important estrogen-induced factor involved in promotion of angiogenesis in breast carcinoma cells, the observation that Glyceollins or elicited soy can suppress VEGF strengthens our central hypothesis that Glyceollins function as anti-estrogens. Other studies have demonstrated that tamoxifen, like estrogen; can induce VEGF expression under certain conditions. The observation that Glyceollin suppresses estrogen induced VEGF and functions as a pure-antagonist like ICI 182,780, lends promise to the possible clinical/dietary utility of Glyceollin/elicited soy in treatment or prevention of breast carcinoma.

During this year we anticipated that the amount required and time to generate sufficient diet for animal studies would be longer than initially proposed. Thus we initiated production of our soy diet ahead of schedule and are in the process of generating diet for animal studies. We also initiated animal validation studies to establish models systems of MCF-7 cells grown *in vivo* for use in the animal studies proposed.

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APPENDIX 1

Burow, Matthew E.

DAMD17-02-1-0596

REGULATION OF ESTROGEN-MEDIATED CELL SURVIVAL BY P160 COACTIVATORS

Christopher B. Weldon M.D., Ph.D.^{2,3,4}, Steven Elliott B.S.^{1,5}, Yun Zhu M.D., Ph.D.^{1,4}, John L. Clayton Ph.D.⁶, Tyler J. Curiel M.D., M.P.H.^{1,4}, Bernard M. Jaffe M.D.^{2,4} and **Matthew E. Burow** Ph.D.^{1,2,4,5}

Departments of Medicine, Section of Hematology & Medical Oncology¹, Surgery², Pharmacology³, The Tulane Cancer Center⁴, The Center for Bioenvironmental Research⁵,
Tulane University Health Sciences Center
New Orleans, Louisiana, 70112 USA
⁶Louisiana State University Health Science Center, LSU Medical School, New Orleans, LA.

Abstract

Background:

The activity and specificity of nuclear/steroid hormone receptors is regulated and dependent upon the type and amount of coactivator proteins (CoA) contained within a cell. However, the role for CoA proteins and their interactions with the ER in controlling apoptotic decisions in breast cancer remains an unexplored area of research.

Methods:

Expression vectors for the p160 CoA genes, NCOA-1 (SRC1), NCOA-2 (GRIP1/TIF2) or NCOA-3 (AIB1/RAC3/ACTR), were transiently transfected into the ER+ breast carcinoma cell line MCF-7. The effects of p160 coactivator expression on cell survival were determined using viability and clonogenic survival assays. The effects of CoA expression on estrogen signaling were assessed using an ERE-luciferase reporter-gene assay. Finally, clonogenic and reporter-gene survival assays were used to examine the molecular inhibition of CoA function (DI-decoy-CoAs) in estrogen-mediated survival in MCF-7 cells.

Results:

Overexpression of three p160-CoA genes enhanced basal and estrogen-mediated expression. They furthermore enhanced cell survival partially by suppressing the effect of TNF-induced cell death, as confirmed by viability and morphological evaluation. NCOA-1 enhancement of cell survival was demonstrated to occur through the suppression of TNF-induced apoptosis with increased long-term clonogenic survival. Expression of DI-NCOA-1 and DI-NCOA-3, but not DI-NCOA-2, decreased clonogenic survival and suppressed estrogen-stimulated colony formation and survival signaling in MCF-7 cells.

Conclusion:

The overexpression of NCOA-1 and NCOA-3 exerted potent survival effects in breast carcinoma cells. Use of dominant inhibitory CoA constructs enhanced TNF-induced cell death and abrogated estrogen-induced clonogenic survival. Molecular inhibition of coactivators represents a mechanism in enhancing sensitivity to chemotherapeutic or hormonal therapy in breast carcinoma.

APPENDIX 2

Burow, Matthew E.

DAMD17-02-1-0596

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE		
Matthew E. Burow, Ph.D.	Assistant Professor of Medicine and Surgery		
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
The University of So. Mississippi, Hattiesburg	B.S.	1989-1994	Biological Sciences
Tulane University, New Orleans, Louisiana	Ph.D.	1994-1998	Mol. & Cellular Biol.
Center for Bioenvironmental Research Tulane University, New Orleans, LA	Post-Doc	1998-2000	Molecular Endocrinology

Positions

1994-1996	Teaching Assistant, Tulane University Medical Center
1998-2000	Post Doctoral Fellow (laboratory of Dr. John McLachlan), Tulane/Xavier Center for Bioenvironmental Research, Tulane University
2000-2002	Research Assistant Professor, Department of Pharmacology, Tulane University Medical School
2002-present	Assistant Professor, Section of Hematology and Medical Oncology, Department of Medicine, Tulane University School of Medicine
2002-present	Adjunct Assistant Professor, Department of Surgery, Tulane University Medical School
2002-present	Program Member, Tulane Cancer Center
2002-present	Program Member, Center for Bioenvironmental Research

Honors, Awards and Other Professional Activities:

1995 – 1997	National Institutes of Health Graduate Pharmacological Sciences Training Grant Fellowship, Department of Pharmacology
1997-2000	US Department of Defense ARMY Breast Cancer Research Fund Pre-Doctoral Fellowship
Member:	American Association for the Advancement of Science (1993- present), American Association for Cancer Research (1998-present), Endocrine Society (2000-present),
Reviewer:	Cancer Research, Journal of Neurochemistry, Histology and Histopathology, Toxicological Sciences, Cellular and Molecular Biology

PUBLICATIONS:

Burow ME, Weldon CB, Tang Y, Navar GL, Krajewski S, Reed JC, Hammond TG, Clejan S, Beckman, BS. Differences in Susceptibility to Tumor Necrosis Factor- α -Induced Apoptosis Among MCF-7 Breast Cancer Cell Variants. *Cancer Res.* 58, 4940-4946 (1998).

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DAMD17-02-1-0596

Boué, S.M., Wiese, T.E., Nehls, S. **Burow, M.E.**, Elliott, S., Carter-Wientjes, C., Shih, B.Y., McLachlan J.A., Cleveland, T.E. Evaluation of the estrogenic effects of legume extracts containing phytoestrogens. *J. of Agriculture and Food Chemistry*. 51(8): 2193-9, (2003).

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Research Support: Current

NIH-NIDDK R01 DK59389-01A2 "PI3K/AKT crosstalk with ER signaling and cell survival"

(Principal Investigator), 02/18/03-02/14/08, \$1,485,000. The objective of this project is to understand the molecular mechanisms and biologic significance of cross-regulation of estrogen receptor (ER) signaling by the phosphatidylinositol-3 kinase (PI3K)-AKT pathway and the role that this has in the development of anti-estrogen resistance in breast cancer.

Department of Defense- U.S.A.M.R.M.C. DAMD-17-02-1-0596 "Potential therapeutic use of glyceollins (I-III), novel anti-estrogenic flavonoid phytochemicals isolated from soy." (Principal Investigator) 07/01/02-06/30/05. Using a nude mouse implanted breast carcinoma model, this proposal will investigate the possible chemopreventive and therapeutic activities of glyceollins (1-3), novel antiestrogenic flavonoids identified from elicited soy.

United States Department of Agriculture Cooperative Agreement # 58-6435-7-019 Period: 3/97-3/04 "Identify Mechanisms of Isoflavonoid Induction in Legumes and their Phytoestrogenic Effects". Collaborative Investigator, (Principal Investigator: John A. McLachlan). Under this agreement, Tulane University will work with the USDA/Agricultural Research Service to conduct research to determine the potency and mechanisms of action of phytoestrogens using cell bioassays, animal systems, and polarized fluorescence spectroscopy.

Completed funding

Department of Defense- U.S.A.M.R.M.C. DAMD-17-01-1-0655 "Coactivator and Corepressor Expression as a Mechanism for Regulation of Apoptosis and Cell Survival in Normal, Immortal and Neoplastic Breast Epithelial Cells" (Principal Investigator) 05/15/01-05/14/03. The primary objective of this proposal is the determine the function that expression levels of specific coactivator and co-repressor proteins have on regulation of apoptosis and cell survival in normal and malignant breast epithelial cells.

Department of Defense- U.S.A.M.R.M.C. DAMD17-97-1-7024 "Effects of Environmental Estrogens on Apoptosis in Normal and Cancerous Breast Epithelial Cells" Principal Investigator 06/01/97-10/31/00 The goals of this project were to identify specific environmental estrogens that suppressed apoptosis in breast epithelial cells and determine if these effects were mediated through increased expression of members of the Bcl-2 family of anti-apoptotic proteins.